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CLINICAL USE OF MONOCLONAL ANTIBODY HuJ591 THERAPY: TARGETING PROSTATE SPECIFIC MEMBRANE ANTIGEN

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ABSTRACT

Purpose: Prostate specific membrane antigen (PSMA) is a cell surface peptidase highly expressed by malignant prostate epithelial cells and vascular endothelial cells of numerous solid tumor malignancies but not normal vascular endothelium in benign tissues or neoplastic epithelial cells of nonprostate malignancies. Monoclonal antibody (mAb) J591 recognizes the extracellular domain of PSMA. The current status of clinical trials using mAb J591 is reviewed.

Material and Methods: The mouse antibody was deimmunized by replacing murine immunoglobulin sequences with human immunoglobulin sequences, resulting in a nonimmunogenic antibody HuJ591. Results of completed and ongoing phase 1 and 2 clinical trials using mAb J591 are presented.

Results: A phase I clinical trial with murine J591 indicated that it possesses high affinity for prostate cancer metastases in bone and soft tissue. A phase I clinical trial showed that HuJ591 was well tolerated, demonstrated excellent targeting to disseminated prostate cancer sites and did not result in a host immune response to the antibody. A phase II clinical trial was initiated to study the efficacy of combining HuJ591 with low dose interleukin-2. Two phase I studies in patients with prostate cancer are in progress using the β-emitting radiometals ^{90}yttrium and ^{177}lutetium linked via a DOTA chelate to HuJ591. Preliminary results from an ongoing phase I trial of ^{111}indium labeled mAb HuJ591 in patients with advanced solid tumors showed that HuJ591 specifically targets nonprostate cancers.

Conclusions: Early results of clinical studies indicate that mAb HuJ591 represents targeted therapy to PSMA and has therapeutic potential in prostate cancer and other urological and solid tumor malignancies.

KEY WORDS: antibodies, monoclonal; prostate-specific antigen; interleukin-2; prostatic neoplasms

During the last 5 years monoclonal antibodies (mAb) have shown great promise in the treatment of a variety of malignancies, including lymphoma, leukemia and breast cancer.[1] There are a number of reasons that prostate cancer represents an excellent target for mAb based therapies. The prostate is a nonessential organ that allows targeting of organ or tissue specific rather than cancer specific, antigens, theoretically resulting in less toxicity. Prostate cancer metastases frequently involve the bone marrow and lymph nodes, which are locations that receive high levels of circulating antibody and have been responsive to mAb therapies in other tumor types (eg lymphoma and breast cancer). mAbs can mediate antitumor effect by activating host immune function or by targeting tumors with conjugated cytotoxins or radioactivity. The availability of a clinical test such as serum prostate specific antigen (PSA) provides an indication for mAb therapy at the first sign of relapse, when disease volume is small and ideally suited for antibody delivery, a point that may be years before clinical manifestations of disease occur. Prostate cancer is radiation sensitive, rendering it an excellent target for radiolabeled mAb therapies. A surrogate marker such as PSA allows rapid clinical evaluation of potential therapeutic efficacy. Although these characteristics suggest that mAb therapy is ideally suited for prostate cancer, the success of such an approach may require the targeted delivery of a cytotoxin or radioactivity in combination with a host immune response.

TARGETING PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA) WITH MONOCLONAL ANTIBODY J591

Prostate specific membrane antigen is a highly restricted prostate epithelial cell membrane antigen.[2,3] The PSMA gene has been cloned, sequenced and mapped to chromosome 11.[4] Although PSMA was originally thought to be prostate specific, recent studies have shown that it is also expressed by small intestine epithelial (brush-border) cells, proximal renal tubule cells and salivary glands. Fortunately, the level of expression in these normal tissues is 100 to 1,000-fold less than in prostate tissue, and these PSMA expressing normal cells are not typically exposed to circulating antibodies due to their brush-border/luminal location. PSMA is expressed in virtually all prostate cancers.[5] Moreover, expression increases in high grade metastatic disease and in hormone refractory prostate cancer.[2,6] In contrast to other prostate related antigens such as PSA, prostatic acid phosphatase and

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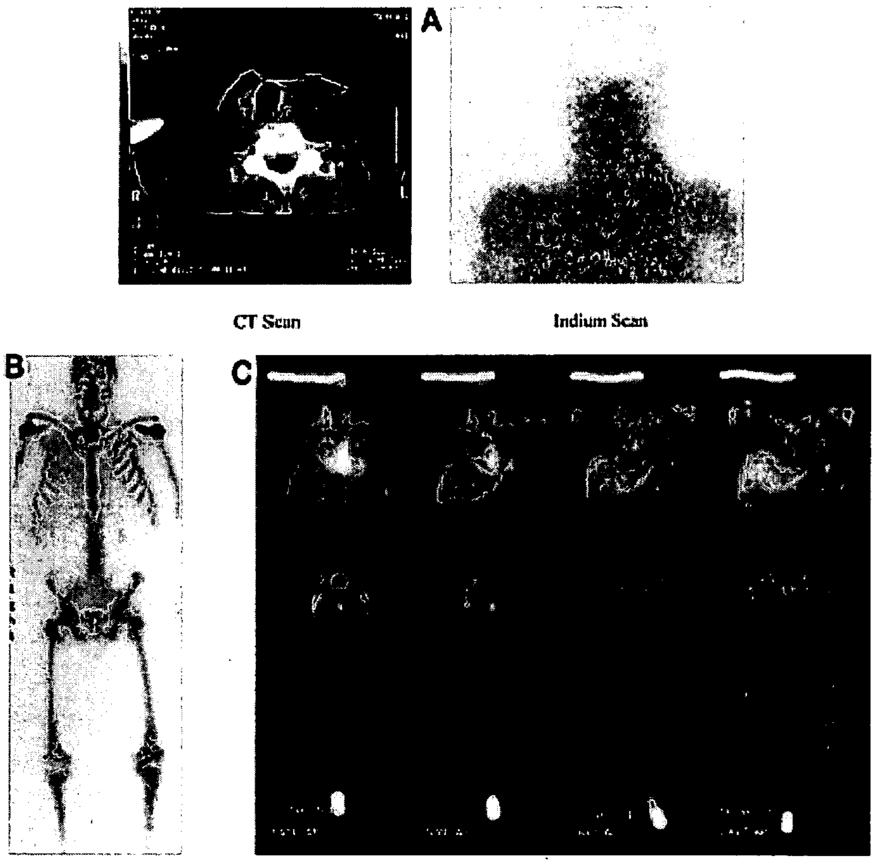

FIG. 1. Imaging of patient with prostate cancer receiving In labeled mAb J591. *A*, supraclavicular lymph node with neuroendocrine features imaged on CT scan and ^{111}In-J591 scan (anterior view). *B*, anterior bone "superscan" shows metastatic prostate cancer to bone. *C*, time course of ^{111}In-J591 scan beginning 1 hour after J591 administration (day 0), with serial images out to 1 week after injection. Day 0 (1 hour) scan reveals isotope primarily in vascular space (heart, great vessels, etc.). Antibody localization can be visualized in femurs and tibias. During ensuing week localization shifts from blood pool into sites of metastatic prostate cancer in bone.

prostate secretory protein, PSMA, which is a type II integral membrane cell surface protein, is not secreted and, therefore, is an excellent target for mAb therapy. PSMA has folate hydrolase and neurocarboxypeptidase activity but its function with respect to prostate cancer has not been defined. Nevertheless, the expression pattern of PSMA makes it an excellent target for mAb therapy for prostate cancer.

Initial validation of PSMA as an in vivo target has been demonstrated by imaging trials with mAb 7E11/CYT-356 (marketed as ProstaScint, Cytogen, Princeton, New Jersey).[7] However, molecular mapping indicates that mAb 7E11/CYT-356 targets an intracellular portion of the PSMA molecule not exposed on the outer cell surface. Since the 7E11/CYT-356 epitope is within the cell, the mAb does not bind viable cells. A mAb to the exposed, extracellular domain of PSMA has the potential of improved localization and enhanced imaging and therapy.[8]

Researchers at our university developed 4 IgG mAbs (J591, J415, J533 and E99) to the extracellular domain of PSMA. In contrast to 7E11/CYT-356, these mAbs recognize 2 distinct noncompeting epitopes located on the exterior of the cell.[8,10] These antibodies demonstrated high affinity binding to prostate cancer cells in tissue culture, on tissue sections and in animal models in vivo.[9,11] Moreover, once bound, PSMA antibody complexes are rapidly internalized with intracellular accumulation of Ab,[9] resulting in the internalization of toxins or isotopes conjugated to the mAb.

Murine monoclonal Ab J591 (muJ591) was chosen for clinical development and has been extensively studied in preclinical models demonstrating that it targets PSMA expressed on LNCaP cells.[9,11] The first clinical trial using J591 (developed by NHB) was completed at New York Presbyterian Hospital-Weill Medical College of Cornell University in 1999. A total of 32 patients with hormone refractory prostate cancer were treated in a phase I biodistribution study of trace labeled ^{131}iodine (I) labeled mAb muJ591. Radioactive iodine was used as it can be linked directly to the immunoglobulin, and there was abundant clinical experience using this isotope. These patients received doses ranging from 0.5 to 200.0 mg conjugated with 10 mCi ^{131}I.

Imaging was performed on the day of treatment (day 0) as well as on days 2, 4 and 6. Known sites of prostate cancer metastases were visualized on the mAb scan in approximately 80% of patients where soft tissue and bony metastatic

sites had been seen by conventional imaging studies (computerized tomography (CT)/magnetic resonance imaging and/or bone scan). Targeting was specific for prostate cancer sites without apparent localization to noncancer sites. In 10 of 21 evaluable patients (48%) a human anti-murine antibody response developed. PSA response was assessed in all patients. Although no patient experienced a major response (greater than 50% decrease in PSA), 1 patient whose PSA was rapidly increasing before therapy experienced stabilization of PSA that lasted greater than 6 months.

A major limitation of mouse mAb J591 in humans is the development of HAMA, which prevents repetitive dosing. Therefore, mAb J591 was de-immunized by a novel method involving specific deletion of epitopes recognized by human B and T cells. De-immunized J591 (HuJ591) was constructed and engineered to possess the additional effect of inducing antibody dependent cellular cytotoxicity with human immune effector cells, with the intent of using HuJ591 as immunotherapy. To determine if HuJ591 could target prostate cancer cells in vivo, Balb C nu/nu mice were first injected in the flank with PSMA expressing LNCaP cells, and tumors were allowed to establish to a size of about 0.8 cm. The animals were subsequently treated with 1 dose of yttrium (^{90}Y) DOTA labeled HuJ591. There was an average reduction in tumor size of 70% in 90% of the treated animals, while no reduction in tumor size was observed in control treated mice (saline or nonradioactive antibody or ^{90}Y-DOTA irrelevant antibody). Imaging studies confirmed that mAb HuJ591 targeted PSMA expressing human prostate cancer xenografts (unpublished data). Several clinical studies have been conducted to investigate the use of HuJ591 in patients with prostate cancer.

CLINICAL TRIALS IN PROSTATE CANCER USING HUJ591

Phase I trial of HuJ591. The initial study using HuJ591 was a phase I trial in patients with progressive prostate cancer. The objectives of this trial were to define the toxicity and maximum tolerated dose of HuJ591, define the pharmacokinetics of HuJ591 and determine the incidence of developing a human antihuman antibody (HAHA) response to HuJ591. Clinical efficacy was evaluated as a secondary end point. Patients received 4 weekly doses of mAb trace labeled with ^{111}Indium (^{111}In) using a DOTA chelate. DOTA can be a molecular "cage" that is covalently linked to the mAb and holds the ^{111}In radiometal. The initial dose levels (week 1) of mAb were 25, 50, 100 and 200 mg/m^2, with the maintenance dose (weeks 2, 3 and 4) at 50% of the initial dose. All patients received a single treatment course. Therapy was well tolerated at all dose levels without toxicity, except for 1 patient who experienced an anaphylactoid reaction due to a rapid infusion rate. Subsequently, the infusion rate was limited to 5 mg per minute, and no further such reactions occurred. No evidence of a HAHA response was detected in any patient.

Total body images were obtained within 1 hour after infusion (day 0) and on days 1, 3 and 5. Sites of prostate cancer metastases that were seen on CT/magnetic resonance imaging and/or bone scan in soft tissue and bone could be imaged with HuJ591 (fig. 1). Excellent imaging could be detected at the lowest dose (25 mg/m^2) of mAb, and no mAb targeting to nonprostate cancer sites was observed. No patient experienced a major clinical response or a 50% or greater decrease in PSA. PSA stabilization during therapy was observed in 3 patients at doses of 25, 100 and 200 mg/m^2. An example of sequential PSA values is illustrated in figure 2.

Phase II trial of HuJ591 in combination with low dose interleukin-2 (IL-2). Interleukin-2 promotes proliferation and enhances the secretory capacity of all major types of lymphocytes, including T cells, B cells and natural killer (NK) cells. Through its effects on NK cells, IL-2 stimulates antigen nonspecific host reactions that involve an interplay between

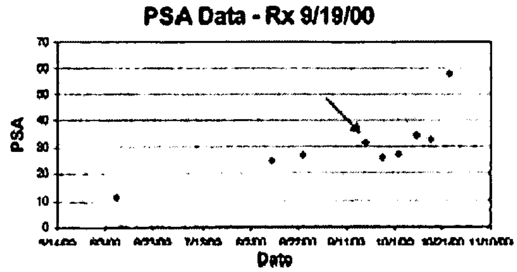

FIG. 2. PSA stabilization with In labeled mAb HuJ591. Patient with progressive prostate cancer and increasing PSA treated with In labeled mAb HuJ591 weekly for 4 weeks. First dose of mAb administered at date at top of figure and indicated by arrow. PSAs were measured each week of mAb administration.

NK cells and monocytes. As a result of these functions, IL-2 should be useful as an immune stimulant, particularly in cancer immunotherapy. Although IL-2 has been used in high doses for the treatment of cancer, researchers examining the immunomodulatory effects and toxicities of continuous low dose IL-2 therapy have demonstrated that doses of IL-2 as low as 1.2 MU daily resulted in the specific expansion of NK cells without any toxicity.[12,13] The selective expansion of human CD3$^-$, CD56$^+$ NK cells during low dose IL-2 began within 2 weeks of therapy and plateaued after 4 to 6 weeks of treatment.[12,14]

IL-2 functions to augment the reticuloendothelial system to recognize antigen antibody complexes by its effects on NK cells and macrophages. By stimulating NK cells to release interferon-γ, granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-α, these cytokines will increase the cell surface density of Fc receptors, as well as the phagocytic capacities of these cells, thereby enhancing the effector arms of humoral and cellular immune responses. The net effect will be to augment the immune response and improve the efficiency of mAb therapy. A small number of clinical trials have combined IL-2 with a mAb.[15-17] In these studies IL-2 was administered intravenously by either bolus or continuous infusion. Toxicity was associated with higher doses of IL-2.

Based on the aforementioned rationale and the ability of

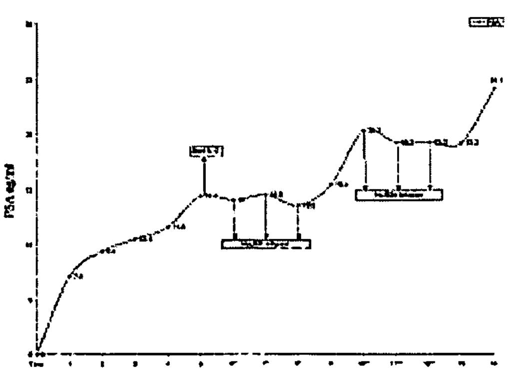

FIG. 3. PSA stabilization with mAb HuJ591 and IL-2. Patient with progressive prostate cancer and increasing PSA treated with low dose IL-2 followed by 3 weekly infusions of mAb HuJ591 (arrows). Asterisks represent weeks of mAb HuJ591 infusions.

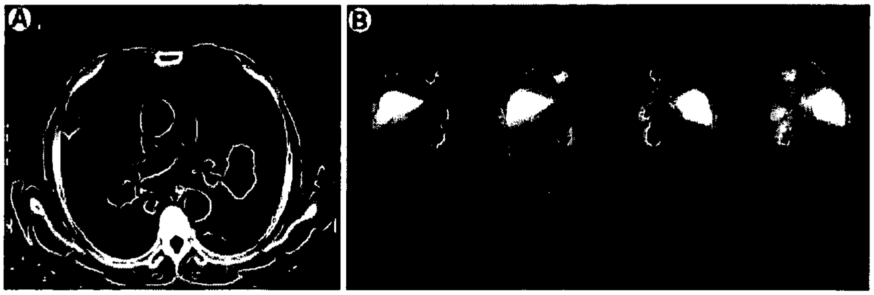

FIG. 4. Imaging of patient with renal cancer receiving In labeled mAb HuJ591. *A*, CT of chest shows lung metastases. *B*, indium scan reveals HuJ591 targeting lung metastases.

mAb HuJ591 to mediate antibody dependent cellular cytotoxicity, a phase II trial of mAb HuJ591 in combination with daily low dose subcutaneous IL-2 was initiated. Patients received daily low dose subcutaneous rIL-2 (1.2×10^6 IU/m^2 daily) continuously beginning on day 1. Following 3 weeks of IL-2 patients received 25 mg/m^2 HuJ591 for 3 consecutive weeks. IL-2 was continued for 2 additional weeks for a total of 8 weeks (1 cycle). Patients who responded to therapy or had stable disease were eligible for additional cycles of therapy. Although it is possible that low dose single agent IL-2 has activity in prostate cancer, the goal of the trial was to study the combination of mAb HuJ591 with IL-2. If significant antitumor activity was observed, a subsequent randomized trial of IL-2 alone versus IL-2 with HuJ591 would be performed. In addition, as it was unclear which stage of prostate cancer would benefit from this approach, 3 distinct cohorts of patients were evaluated: 1) biochemical relapse, hormone naive (ie increasing PSA following radical prostatectomy or radiation therapy, without evidence of metastatic disease); 2) biochemical relapse, hormone refractory (ie increasing PSA following hormonal therapy without prior chemotherapy and with or without radiographically documented metastatic disease); and 3) hormone refractory (having received prior chemotherapy). Flow cytometric analysis of peripheral blood mononuclear cells for lymphocyte subsets and NK cells was performed to confirm that the desired immune effects of IL-2 occurred.

More than 15 patients have been treated to date. Toxicity has been mild, consisting of erythema at the injection site, asymptomatic thyroid function test abnormalities, occasional mild flu-like symptoms and eosinophilia, which is commonly associated with IL-2. Other toxicities observed in patients receiving high dose IL-2, such as hypotension, renal failure, respiratory distress or neurological symptoms, were not observed. Flow cytometric studies confirmed significant expansion of the NK cell population. Numerous patients have received more than 1 cycle. Stabilization of PSA was observed (fig. 3). These findings were similar to those in the phase I study. Taken together, these 2 studies indicated that weekly administered HuJ591 was well tolerated, specifically targeted prostate cancer and appeared to result in PSA stabilization in some patients.

Phase I trials of radiolabeled HuJ591. Based on the success of mAbs in targeting radiation therapy directly to tumor cells in other malignancies, 2 phase I clinical trials were initiated in patients with hormone refractory prostate cancer using the β-emitting radiometals, ^{90}Y and ^{177}lutetium (^{177}Lu), linked to HuJ591 via a DOTA chelate. In contrast to iodine, a chelator is required to attach these isotopes to an immunoglobulin. A major disadvantage of ^{131}I is that the isotope is cleaved from the immunoglobulin following internalization of the isotope antibody complex and then diffuses passively out of the cell. In contrast, ^{90}Y and ^{177}Lu are effectively irreversibly trapped within the cell. ^{90}Y and ^{177}Lu were studied because of their different characteristics and to determine if 1 isotope may have greater antitumor activity in treating prostate cancer. ^{90}Y has a shorter half-life (about 2.5 days), higher energy and longer path range, whereas ^{177}Lu possesses a longer half-life (about 6.7 days), lower energy and shorter path range.

The primary objectives of these independent trials were to define the maximum tolerated dose, dosimetry, pharmacokinetics and immunogenicity of the ^{90}Y and ^{177}Lu-mAb conjugates. Antitumor responses were assessed as a secondary end point. Dose levels were escalated in cohorts of 3 to 7 patients with a 6 to 8-week observation period between dose levels. All patients underwent nuclear imaging with either ^{111}In-HuJ591 (patients receiving ^{90}Y, a pure β emitter that does not image on radionuclide scans) or ^{177}Lu-HuJ591 (γ-emitting properties), and the imaging results were compared to standard imaging studies. Preliminary analysis indicated that bone and soft tissue metastases seen on conventional bone scans and CT were accurately targeted by HuJ591 in virtually every patient. No patient has had HAHA in either study. The dose limiting toxicity of myelosuppression in the ^{90}Y-HuJ591 study occurred at a dose of 20mCi/m^2 and the ^{177}Lu-HuJ591 is ongoing, with the dose limiting toxicity not yet reached. Dose related antitumor effects have been noted including PSA and measurable disease responses.

Clinical trials of other solid tumor malignancies using HuJ591. Immunohistochemical studies have shown that PSMA is expressed not only by prostate epithelial cells, but also by vascular endothelial cells of numerous solid tumor malignancies. However, PSMA is not expressed by normal vascular endothelium in benign tissues or neoplastic epithelial cells of nonprostate malignancies.[8, 18] Localization of the anti-PSMA mAbs to tumor associated neovasculature was confirmed by CD34 immunostaining. A more recent analysis of PSMA expression, using reverse transcription polymerase chain reaction and in situ hybridization, similarly demonstrated mRNA transcripts for PSMA in the endothelium of tumor associated neovasculature of multiple nonprostatic solid tumor malignancies.[19] These data suggest that PSMA may be an effective target for mAb based antineovasculature therapy. Therefore, we initiated a phase I dose-escalation trial of ^{111}I labeled mAb HuJ591 to test the hypothesis that PSMA is a target for HuJ591 vasculotoxic therapy, define HuJ591 maximum tolerated dose and toxicity in patients without prostate cancer determine pharmacokinetics and biodistribution of HuJ591, and assay for the development of HAHA. Eligible patients included those with refractory solid tumor malignancies that expressed PSMA on the neovasculature. Patients entering the study had a variety of solid tumors, including renal, bladder, colon, pancreatic, breast

and lung. Patients with these tumors were similar to those with prostate cancer in that they tolerated mAb HuJ591 well with no development of HAHA. ^{111}In-J591 scanning showed localization of J591 to tumor sites in 15 of 19 patients (79%), which occurred in metastatic sites in viscera, soft tissue and bone (fig. 4). No objective clinical responses have yet occurred in this ongoing phase I study.

CONCLUSIONS

PSMA represents an ideal cell surface protein for targeted therapy of cancer cells in prostate cancer and vascular endothelial cells in other solid tumors. Clinical trials using monoclonal antibody J591, which recognizes the extracellular domain of PSMA, indicate that this antibody can effectively target prostate cancer and other solid tumor malignancies, including kidney and bladder cancers. Furthermore, J591 can be used to deliver radioactivity or other toxins to metastatic prostate cancer and neoplasia associated vascular endothelium. Ongoing and future clinical trials will define the therapeutic role of J591 in urological cancers.

J591 was patented by the Cornell Research Foundation and licensed to BZL Biologics, Inc., Framingham, Massachusetts. Maureen Joyce, Alyssa Rosmarrin, Marta Cobham and the nursing staff of the Clinical Research Center assisted with the study, and Juan Pena provided data management.

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DISCUSSION

Dr. Joel B. Nelson. You showed that the PSA stabilized and then increased. Do you know if the antibody directly interacts with the PSA assay? For example, if you spike serum, can you change the assay results?

Dr. David Nanus. I believe that has been done, and the answer is no.

Dr. William R. Sellers. Keeping in mind that this is targeted therapy, do you have any idea, either by imaging or other means, at what dose the receptors are saturated? You can imagine that toxicity is the spillover or effective higher dose and that if you gave multiple smaller doses sufficient to saturate the tumor, then that is all you would need.

Doctor Nanus. In the yttrium study the dose at which we saw myelosuppression was probably not related to targeting but to radioactivity. We are in the range of the maximum tolerated dose of yttrium observed in other trials using different antibodies. We are not even sure whether bone marrow involvement would matter. We have had more experience with re-treatment and are moving forward with the higher doses of lutetium. But we are also planning multiple treatments at a lower dose where we had little toxicity. We still do not know if toxicity will be cumulative.

Doctor Sellers. Do you have an imaging agent that shows your drug binding to the cancer? Can you use those data at different doses?

Doctor Nanus. Yttrium does not image, and patients receive indium labeled antibody to image the tumor. In the lutetium study almost all cases imaged, even at low doses of radioactivity. At the lowest doses we saw no biological activity, no responses, in the yttrium study. Responses in soft tissue were seen at higher doses of radioactivity. Keep in mind that this is a phase 1 study. It is unclear if repeat lower dosing will result in more biological activity.

Dr. Mack Roach, III. When you are looking at radiation responses you should be a little careful because when you radiate the prostate, the half-life of PSA is about 3 months for someone with an intact prostate. If you are doing studies using an injection and then follow the PSA you may not see the effect. If you draw PSAs every week during the course of external beam radiotherapy the PSA bounces, and it could take several months to get a response. So it is possible that you could be missing some responses that could be occurring later if you were giving radiation in a different way. Are you stratifying cases according to presence or absence of the prostate? When you give radioactive iodine to patients with thyroid cancer, you first destroy the thyroid, because it will suck up a lot of the iodine. In patients with an intact prostate it is possible that the prostate is taking up a lot of your antibody.

Doctor Nanus. We are not stratifying patients for the presence of a prostate gland but we are imaging patients, and identifying and measuring where the antibody targets. You raise an interesting point. I know of 1 patient whose PSA was 3,500 and he received 1 dose of yttrium. After 6 months we did not think he had a clinical response but the PSA had decreased. He is off the study. According to protocol, the patients must be followed for 12 weeks from the time of antibody administration for response.

Doctor Roach. What if you gave a patient a week of external beam radiation and then stopped, waited a while and gave him a bit more and stopped again? We have to finish the entire course. I agree that it is possible that giving lower doses continuously for longer periods would get a response that might take longer to be seen but it might be a more sustained response.

Dr. Philip W. Kantoff. Your big concern in doing that would be collateral damage to the bone marrow.

Doctor Roach. That is why you give lower doses.

Dr. William K. Oh. Normally, we think of immediate responses in hormone refractory disease. I do not know if there are data on samarium. Samarium and strontium sometimes make the PSA decrease but I have never seen any good data on the time course. We are used to seeing responses within a few weeks and making a judgment as to chemotherapy.

Dr. Matthew R. Smith. I have been led to believe that the slow kinetics of PSA decrease is due to the slow rate of growth in the primary tumor. Does the fact that these are presumably faster growing tumors have any bearing on your comment?

Doctor Roach. The tumors in the patients we radiate are not growing that fast.

Doctor Oh. Is this an angiogenesis inhibitor?

Doctor Nanus. We have preliminarily performed corneal pocket assays using purified PSMA and found no effect in basic fibroblast growth factor mediated angiogenesis. However, PSMA is not the only cell surface peptidase whose expression is increased in vascular endothelial cells in malignancies. Aminopeptidase A and aminopeptidase N have been shown to have increased expression in vascular endothelial cells. My theory is that something is turning on these peptidases so that they either activate a proangiogenic peptide or inactivate an antiangiogenic peptide. We are in the process of trying to determine what the PSMA is doing but unfortunately there are no good animal models.

Exhibit B

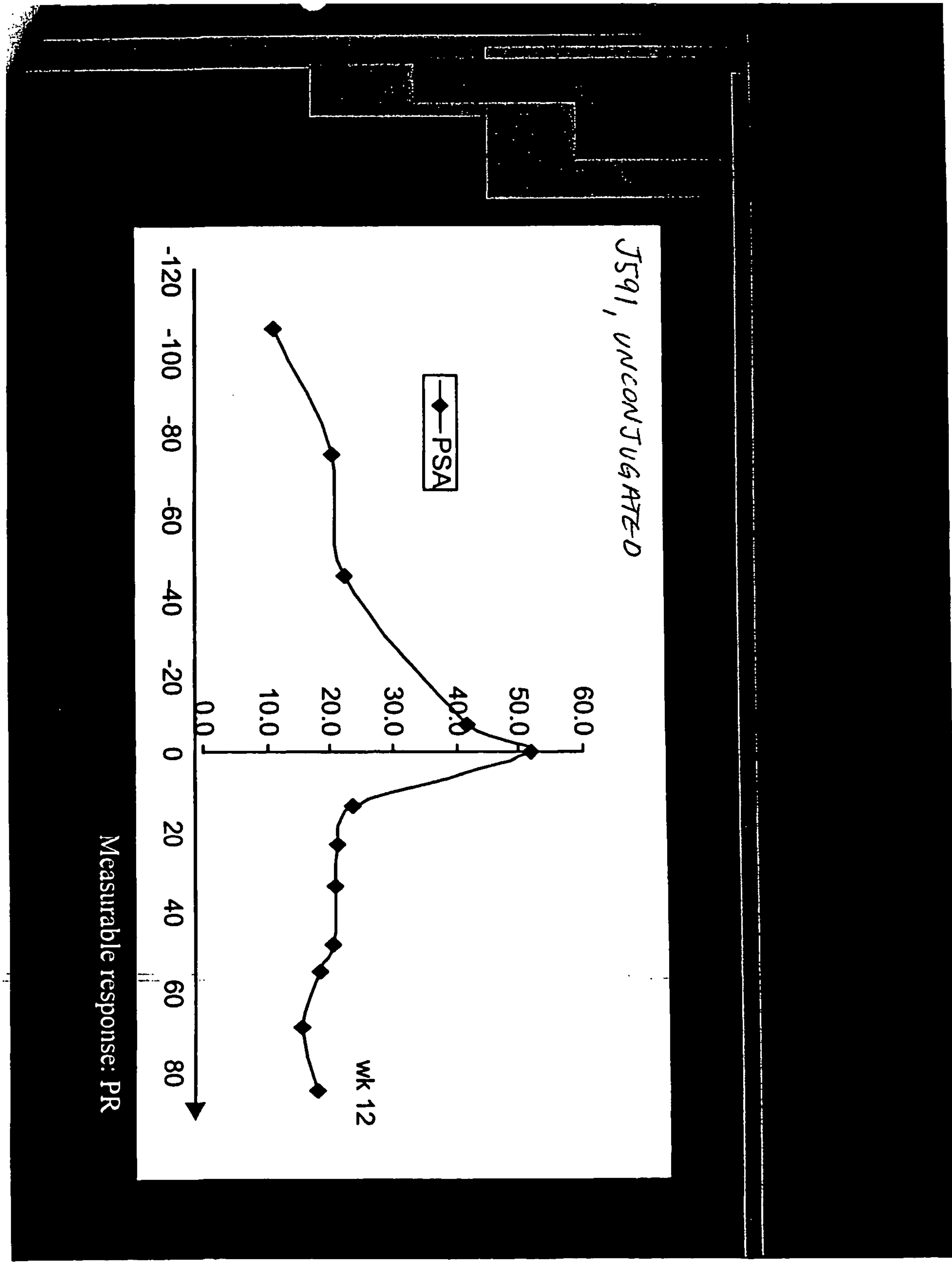
J591, UNCONJUGATED
PSA
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-120
-100
-80
-60
-40
-20
0
20
40
60
80
wk 12
Measurable response: PR

Exhibit A

Localization: Renal Cell (5mg)